

Original article

# Chemical composition and biological activities of the leaves essential oil of *Alphonsea monogyna* collected in Hue city

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## Abstract

**Background:** *Alphonsea monogyna* (Roxb.) Hook.f. & Thomson belongs to the genus *Alphonsea*, distributed in Southeast Asian countries. Information on the chemical composition and biological properties of *A. monogyna* remains limited. This study aimed to investigate the chemical composition of the leaf essential oil of *A. monogyna* and evaluate its antioxidant, anti-inflammatory, and antibacterial activities.

**Materials and Methods:** The essential oil was extracted by hydrodistillation and analyzed by GC–MS. Antioxidant activity was assessed by the ABTS<sup>•+</sup> assay, nitric oxide (NO) inhibition was determined in LPS-stimulated RAW 264.7 macrophages, and antibacterial activity was evaluated using the broth microdilution method.

**Results:** A total of 47 compounds were identified, accounting for 99.21% of the total oil, dominated by sesquiterpene hydrocarbons (50.99%) and oxygenated sesquiterpenes (36.84%). The main constituents were guaiol (13.48%), germacrene D (10.31%), bulnesol (9.85%), and bicyclogermacrene (8.26%). The essential oil exhibited moderate antioxidant activity ( $IC_{50} = 61.64 \pm 1.13 \mu\text{g/mL}$ ), significant NO inhibitory effect ( $IC_{50} = 56.35 \pm 2.57 \mu\text{g/mL}$ ), and selective antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus fermentum*).

**Conclusion:** The essential oil of *A. monogyna* demonstrates promising antioxidant, anti-inflammatory, and antibacterial potentials, suggesting its value as a natural source of bioactive compounds for pharmaceutical applications.

**Keywords:** *Alphonsea monogyna*; essential oil; chemical composition; antioxidant; anti-inflammatory; antibacterial.

## 1. BACKGROUND

The genus *Alphonsea* (family Annonaceae) comprises approximately 30 species distributed throughout tropical and subtropical Asia, including Vietnam, Malaysia, India, and Thailand. Many species of this genus have been used in traditional medicine for the treatment of fever, inflammation, infections, and other disorders. Phytochemical studies to date have revealed that *Alphonsea* species contain a wide range of secondary metabolites such as alkaloids, flavonoids, essential oils, steroids, tannins, and saponins [1]. Essential oils obtained from different parts of *A. philastreana*, *A. gaudichaudiana*, and *A. elliptica* have been found to be rich in sesquiterpenes including (E)- $\beta$ -ocimene,  $\beta$ -caryophyllene,  $\alpha$ -humulene, bicyclogermacrene, and guaiol [2,3]. Alkaloids represent a major class of constituents in the genus, with several aporphine, oxoaporphine, and azafluorenone derivatives such as liriodenine, atherospermidine, kinabaline, and N-methylouregidione identified

from *A. elliptica*, *A. cylindrica*, and *A. sclerocarpa* [4–6]. Flavonoids such as vitexin, isovitexin, orientin, and kaempferol have also been reported, together with triterpenoids and steroids including stigmast-22-en-3-one and stigmasta-4,6,22-trien-3-one [3,7]. These compounds have been associated with diverse biological properties, including antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities. Among the known biological effects, several *Alphonsea* extracts and isolated compounds have demonstrated antioxidant potential through DPPH and FRAP assays, anti-inflammatory activity via inhibition of cyclooxygenase-2 (COX-2) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production, and cytotoxicity against human cancer cell lines such as HepG2, MCF-7, and A549 [6,8].

*Alphonsea monogyna* (Roxb.) Hook.f. & Thomson is a native species distributed in Vietnam and several Southeast Asian countries [9]. Preliminary observations indicate that this species produces a distinct aroma and possesses traditional medicinal

applications, suggesting the presence of essential oils and other bioactive secondary metabolites [1,10]. However, to date, there have been very few reports on the chemical composition or biological activities of *A. monogyna*, particularly concerning its volatile constituents.

Therefore, this study aims to investigate the chemical composition of the essential oil from the leaves of *A. monogyna* collected in Hue City and to evaluate its antioxidant, anti-inflammatory, and antibacterial activities. The findings are expected to contribute to the understanding of the chemical diversity and pharmacological potential of the *Alphonsea* genus and provide a scientific basis for the future utilization of *A. monogyna* as a potential source of bioactive natural products.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials

Leaves (1.0 kg) of *A. monogyna* were collected in Hue City, Vietnam, in November 2024. The species was identified by Dr. Le Tuan Anh from the Vietnam National Museum of Nature. A voucher specimen (AM-T234) has been deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Hue University, Vietnam.

### 2.2. Cell Source and Culture

The RAW 264.7 macrophage cell line was kindly provided by Prof. Dr. Domenico Delfino, University of Perugia, Italy. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (FBS; GIBCO), 10 mM HEPES, and 1.0 mM sodium pyruvate. The cells were passaged at a 1:3 ratio every 3-5 days and cultured in a CO<sub>2</sub> incubator under stable conditions of 37°C with 5% CO<sub>2</sub>.

### 2.3. Methods

#### 2.3.1. Hydrodistillation of the essential oil

Following methods established in previous work [11] prior to the hydrodistillation process, the fresh leaves and stems of *I. rhodantha* were shredded. Samples were then subjected to hydrodistillation using a Clevenger apparatus for three hours at normal pressure, according to the Vietnamese Pharmacopoeia V (2019) [12]. The essential oils were then collected, removed water by using sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), and stored at 4°C in a refrigerator. The experiments were performed in triplicate.

#### 2.3.2. Analysis of the essential oil

The essential oil composition was analyzed by GC-MS using a Shimadzu GC-MS-QP2010 Plus system equipped with an Equity-5 capillary column (30 m ×

0.25 mm, 0.25 μm film). The oil was diluted 1:100 in n-hexane, and 1 μL was injected in splitless mode with helium (1.5 mL/min) as the carrier gas. The oven was programmed from 60°C (2 min) to 240°C at 3°C/min (10 min hold), then to 280°C at 5°C/min (40 min hold); injector and interface temperatures were 280°C. Mass spectra were recorded at 70 eV (45–500 m/z, 1 scan/s). Compound identification was based on comparison of mass spectra and retention indices with literature data [13], GC/MS libraries (NIST 2018, WILEY 7), and co-injection of standards. Quantification was performed using relative peak area percentages.

#### 2.3.3. ABTS•<sup>+</sup> Radical Scavenging Activity

The ABTS•<sup>+</sup> radical cation was generated by reacting 7 mM ABTS solution with 2.4 mM potassium persulfate in distilled water at a 1:1 ratio. The mixture was left to stand in the dark at room temperature for approximately 14 hours to allow complete radical formation. The resulting solution was then diluted with methanol until its absorbance reached 0.83 ± 0.01 at 734 nm.

For the antioxidant assay, 2 mL of the diluted ABTS•<sup>+</sup> solution was combined with 2 mL of the sample solution at different concentrations and kept in the dark for 7 minutes. The absorbance of each mixture was measured at 734 nm using methanol as the blank and ascorbic acid as the reference antioxidant.

The radical scavenging capacity was determined as the percentage of inhibition (%) according to the equation: %I = [(A<sub>control</sub> - A<sub>sample</sub>) / A<sub>control</sub>] × 100,

where A<sub>control</sub> is the absorbance of the ABTS•<sup>+</sup> solution without sample and A<sub>sample</sub> is that of the reaction mixture containing the test sample. The IC<sub>50</sub> value, representing the concentration required to neutralize 50% of the radicals, was derived from the corresponding dose-response curve. All measurements were carried out in triplicate, and the data are presented as mean ± standard deviation (SD). [14].

#### 2.3.4. NO Inhibition Assay

To evaluate the inhibition of nitric oxide (NO) production, RAW 264.7 cells were seeded in 96-well plates at a density of 2 × 10<sup>5</sup> cells/well and incubated for 24 hours at 37°C with 5% CO<sub>2</sub>. The medium was replaced with DMEM without FBS, and 1 μg/mL lipopolysaccharide (LPS) was added to stimulate NO production. Test samples were added to the wells at varying concentrations (100, 20, 4, and 0.8 μg/mL), followed by a 24-hour incubation under the same conditions. In this study, Dexamethasone

(Sigma) served as the positive control, and dimethyl sulfoxide diluted to a concentration of 1.0% served as the negative control. NO production was indirectly quantified by measuring nitrite concentrations using the Griess reagent (Promega Corporation, WI, USA). For this, 100  $\mu$ L of the culture supernatant was transferred to a new 96-well plate and mixed with 100  $\mu$ L of Griess reagent, consisting of 50  $\mu$ L of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 50  $\mu$ L of 0.1% (w/v) N-1-naphthylethylenediamine dihydrochloride. The mixture was incubated for 10 minutes at room temperature, and the absorbance was measured at 540 nm using a BioTek Elx800 microplate reader. The IC<sub>50</sub> (50% inhibitory concentration) values were calculated using TableCurve 4.0 software (Systat Software Inc., USA) [15].

### 2.3.5. MTT cell viability assay

To assess cell viability, the 96-well plate used for the NO inhibition assay was supplemented with 90  $\mu$ L of fresh DMEM and 10  $\mu$ L of MTT solution (final concentration: 5 mg/mL) per well. The plate was incubated for 4 hours at 37°C. Subsequently, the medium was removed, and the resulting formazan crystals were dissolved by adding 50  $\mu$ L of 100% DMSO per well. Absorbance was measured at 540 nm using a BioTek Elx800 microplate reader. Cell viability in the presence of the test substances was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{OD(\text{sample}) - OD(\text{blank})}{OD(\text{DMSO}) - OD(\text{blank})}$$

### 2.3.6. Antimicrobial activity

The antimicrobial activity of the test samples was evaluated through broth dilution method to determine their inhibitory effects on microbial growth [16,17]. The antibacterial activity was evaluated using six representative bacterial strains: *Bacillus*

*subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 13709), *Lactobacillus fermentum* (N4), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), and *Salmonella enterica*. Bacterial cultures were maintained on Mueller–Hinton Broth (MHB) and Mueller–Hinton Agar (MHA) for general cultivation, while Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) were employed for aerobic bacterial growth.

The test samples were serially diluted in 100% DMSO and sterile distilled water to prepare a series of 4–10 concentrations. Activated bacterial suspensions were standardized to  $5 \times 10^5$  CFU/mL prior to testing. For each assay, 10  $\mu$ L of the diluted test sample was added to 96-well microplates, followed by 190  $\mu$ L of the bacterial suspension. The microplates were incubated at 37°C for 16–24 hours.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the sample that completely suppressed visible bacterial growth. The half-maximal inhibitory concentration (IC<sub>50</sub>) was calculated from the percentage of growth inhibition at varying concentrations. Ampicillin and Cefotaxime served as positive controls for Gram-positive and Gram-negative bacteria, respectively.

### 2.4. Statistical analysis

All experiments were performed in triplicate, and results are presented as mean  $\pm$  standard deviation (SD).

## 3. RESULTS

### 3.1. Chemical composition of essential oil

The essential oil was lighter than water, pale yellow in color, and possessed an aromatic scent. The yield of the oil was  $0.25 \pm 0.05\%$  based on the fresh weight. The chemical composition of the leaf essential oil of *Alphonsea monogyna* is presented in Table 1.

**Table 1.** Chemical constituents of the leaf essential oil of *Alphonsea monogyna*.

No.	RT <sup>a</sup>	Compounds	RI <sup>b</sup>	RI <sup>c</sup>	Concentration (%)
1	6.69	$\alpha$ -Pinene*	932	927	1.50
2	7.98	Sabinene	969	968	0.22
3	8.13	$\beta$ -Pinene*	974	973	2.85
4	8.58	Myrcene*	988	987	0.36
5	9.09	$\alpha$ -Phellandrene	1002	1003	1.12
6	10.08	<b><math>\beta</math>-Phellandrene</b>	1025	1028	<b>4.36</b>
7	10.79	(E)- $\beta$ -Ocimene	1044	1045	0.67
8	16.35	Terpinen-4-ol	1174	1176	0.15
9	23.04	$\delta$ -Elemene	1335	1328	1.52
10	23.63	<b>Bicycloelemene</b>	1345	1342	<b>8.39</b>
11	24.91	$\alpha$ -Ylangene	1373	1372	0.35

12	25.10	$\alpha$ -Copaene	1374	1376	0.26
13	25.85	$\beta$ -Elemene	1389	1394	2.64
14	27.11	<b>Aromadendrene</b>	1419	1424	<b>7.34</b>
15	27.46	$\alpha$ -Cubebene	1432	1433	2.63
16	27.57	$\gamma$ -Elemene	1434	1435	0.78
17	27.78	$\alpha$ -Guaiene	1437	1441	0.65
18	28.04	$\gamma$ -Muurolene	1451	1447	1.43
19	28.19	<i>allo</i> -Aromadendrene	1458	1450	0.25
20	28.44	$\beta$ -Caryophyllene*	1456	1456	2.36
21	28.65	dehydro-Aromadendrane	1460	1462	0.29
22	28.81	Cadina-1(6),4-diene< <i>cis</i> ->	1461	1466	0.65
23	29.76	<b>Germacrene-D</b>	1483	1489	<b>10.31</b>
24	29.85	$\beta$ -Selinene	1489	1491	0,63
25	30.38	<b>Bicyclgermacrene</b>	1500	1504	<b>8.26</b>
26	30.58	$\alpha$ -Bulnesene	1509	1509	1.28
27	30.84	$\gamma$ -Cadinene	1513	1516	0.07
28	31.12	$\beta$ -Elemol	1530	1523	0.21
29	31.22	$\delta$ -Cadinene	1522	1525	0.68
30	31.55	Zonarene	1528	1534	0.08
31	32.37	<b>Elemol</b>	1548	1555	<b>4.47</b>
32	32.57	Germacrene B	1559	1558	0.14
33	32.80	( <i>E</i> )-Nerolidol	1561	1566	0.40
34	33.36	Spathulenol	1577	1580	0.77
35	33.60	Globulol	1590	1586	0.73
36	33.92	Viridiflorol	1592	1594	0.29
37	34.52	<b>Guaiol</b>	1600	1610	<b>13.48</b>
38	34.74	Rosifoliol	1600	1616	1.23
39	35.06	10-epi- $\gamma$ -Eudesmol	1622	1624	0.40
40	35.46	$\gamma$ -Eudesmol	1630	1635	0.46
41	35.85	Agarospinol	1646	1646	0.72
42	36.02	$\alpha$ -Muurolol	1644	1650	0.44
43	36.13	$\beta$ -Eudesmol	1649	1653	0.37
44	36.30	Pogostol	1651	1658	0.77
45	36.44	$\alpha$ -Eudesmol	1652	1661	2.24
46	37.06	<b>Bulnesol</b>	1670	1678	<b>9.85</b>
47	51.48	Phytol	2118	2112	0.16
<b>Monoterpene hydrocarbon (M)</b>					<b>11.08</b>
<b>Oxygenated monoterpene (OM)</b>					<b>0.15</b>
<b>Serquiterpene hydrocarbon (S)</b>					<b>50.99</b>
<b>Oxygenated serquiterpene (OS)</b>					<b>36.84</b>
<b>Others (O)</b>					<b>0.16</b>
<b>Total</b>					<b>99.21</b>

Note: RT: Retention time; [a] Elution order on Equity-5 column; [b] Retention indices on Equity-5 column; [c] Literature retention indices [41]. All components that were found in the essential oils at

greater than 4.0% were highlighted with bold letters. \*co-injection with authentic samples.

### 3.2. Antioxidant activity

The antioxidant potential of *A. monogyna* was

assessed using the ABTS<sup>•+</sup> radical scavenging assay, with ascorbic acid employed as the positive standard. The essential oil of *A. monogyna* demonstrated notable free radical scavenging activity, exhibiting an IC<sub>50</sub> of 61.64 ± 1.13 µg/mL, while ascorbic acid had an IC<sub>50</sub> of 1.27 ± 0.08 µg/mL.

### 3.3. Inhibitory activity on NO production

The inhibitory effect of *A. monogyna* leaf essential oil on nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages is summarized in Table 2. The essential oil exhibited concentration-dependent NO inhibition, with IC<sub>50</sub> values of 56.35

± 2.57 µg/mL, which were weaker than that of the reference drug dexamethasone (IC<sub>50</sub> = 14.41 ± 1.55 µg/mL). The essential oil also demonstrated high cell viability within the tested concentration range. Even at 100 µg/mL, cell viability remained at 92.36 ± 1.84%, slightly lower than that of dexamethasone (99.71 ± 6.18%), indicating negligible cytotoxicity at high concentrations. These findings suggest that the *A. monogyna* essential oil exhibits appreciable NO inhibitory activity with a favorable safety profile, supporting its potential as a natural anti-inflammatory agent.

**Table 2.** NO inhibitory activity of *A. monogyna* leaves essential oil.

Concentration (µg/mL)	<i>A. monogyna</i>		Dexamethasone	
	% I	% CS	% I	% CS
100	90.74 ± 2.65	92.36 ± 1.84	84.26 ± 1.84	99.71 ± 6.18
20	16.08 ± 1.54	99.72 ± 0.39	52.29 ± 1.08	103.4 ± 7.28
4	8.32 ± 0.75		42.26 ± 0.93	
0.8	2.68 ± 0.30		31.88 ± 1.02	
<b>IC<sub>50</sub></b>	<b>56.35 ± 2.57</b>		<b>14.41 ± 1.55</b>	

Note. Data are shown as mean ± standard deviation (n = 3); % I: % inhibition; % CS: % cell survival

### 3.4. Antimicrobial activity

The antibacterial activity of *A. monogyna* leaf essential oil is presented in Table 3. The oil exhibited selective inhibitory effects against Gram-positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Lactobacillus fermentum*, with the strongest activity observed against *L. fermentum* (IC<sub>50</sub> = 117.00 ± 7.37 µg/mL). In contrast, the oil showed negligible inhibitory effects on Gram-negative strains (*Salmonella enterica*, *Escherichia coli*, and *Pseudomonas aeruginosa*), indicating a selective antibacterial spectrum typical of sesquiterpenoid-rich essential oils.

**Table 3.** Antimicrobial activity of essential oil from the leaves of *A. monogyna*.

Samples	Concentration (µg/mL)	Gram-positive			Gram-negative		
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>L. fermentum</i>	<i>S. enterica</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
<i>A. monogyna</i>	256	100	100	100	0	0	0
	64	0	0	31	0	0	0
	16	0	0	0	0	0	0
	4	0	0	0	0	0	0
	1	0	0	0	0	0	0
	<b>IC<sub>50</sub></b>	<b>160.00 ± 6.68</b>	<b>160.00 ± 7.89</b>	<b>117.00 ± 7.37</b>	> 256	> 256	> 256
	<b>MIC</b>	<b>256 ± 0.00</b>	<b>256 ± 0.00</b>	<b>256 ± 0.00</b>	> 256	> 256	> 256
Ampicillin	<b>IC<sub>50</sub></b>	0.02 ± 0.005	3.62 ± 0.15	1.03 ± 0.07			
	<b>MIC</b>	0.125 ± 0.00	32 ± 0.00	32 ± 0.00			
Cefotaxime	<b>IC<sub>50</sub></b>				0.43 ± 0.05	0.007 ± 0.002	4.34 ± 0.15
	<b>MIC</b>				32 ± 0.00	0.5 ± 0.00	8 ± 0.00

#### 4. DISCUSSIONS

From the leaf essential oil of *Alphonsea monogyna*, a total of 47 compounds were identified, accounting for 99.21% of the total oil composition. The major constituents included guaiol (13.48%), germacrene D (10.31%), bulnesol (9.85%), bicyclogermacrene (8.26%), aromadendrene (7.34%), elemol (4.47%), and  $\beta$ -phellandrene (4.36%). The essential oil was characterized by a high proportion of sesquiterpenes, particularly sesquiterpene hydrocarbons (50.99%), followed by oxygenated sesquiterpenes (36.84%) and monoterpene hydrocarbons (11.08%).

The chemical composition of *A. monogyna* essential oil in this study showed remarkable differences compared to that reported by Quang et al. [10]. Although both samples were rich in sesquiterpenes, the relative proportions of compound groups varied notably. Specifically, the present sample contained 50.99% sesquiterpene hydrocarbons and 36.84% oxygenated sesquiterpenes, whereas the previously reported oil comprised 73.5% sesquiterpene hydrocarbons and only 4% oxygenated forms. These differences may result from variations in geographical origin, harvest time, plant growth stage, or extraction and analytical conditions. Despite such fluctuations, both studies identified several common constituents, germacrene D, bicyclogermacrene, and  $\beta$ -caryophyllene, though their relative contents differed substantially. In particular,  $\beta$ -caryophyllene (13.8%) and  $\delta$ -cadinene (12.5%) were the predominant compounds in the earlier study, whereas guaiol (13.48%), germacrene D (10.31%), and bulnesol (9.85%) were dominant in the present investigation. These compositional variations highlight the chemical plasticity of *A. monogyna* essential oil and emphasize the necessity for further studies to assess the influence of ecological and technical factors on its composition and quality.

Additionally, the chemical profile of *A. monogyna* differed substantially from other *Alphonsea* species, reflecting the chemical diversity within this genus. Guaiol, the principal compound in *A. monogyna*, was not a dominant constituent in *A. philastreana*, *A. gaudichaudiana*, *A. tonkinensis*, or *A. elliptica* [1,18]. In these species, guaiol accounted for only 9.0% in *A. philastreana* and 5.2% in *A. gaudichaudiana*, markedly lower than that in *A. monogyna* [19]. Similarly, germacrene D (10.31%) was a major constituent of *A. monogyna*, but its levels were modest in *A. tonkinensis* (6.3%) and *A. elliptica* (7.2%) and much lower in *A. philastreana* and *A. gaudichaudiana* [19,20].

The results demonstrated that the leaf essential oil of *A. monogyna* exhibits a broad spectrum of biological activities, including antioxidant, antibacterial, and anti-inflammatory effects. Regarding antioxidant activity, the essential oil of *A. monogyna* showed a moderate ability to scavenge ABTS $\bullet^+$  free radicals with an IC<sub>50</sub> value of 61.64  $\pm$  1.13  $\mu$ g/mL, compared to that of ascorbic acid (IC<sub>50</sub> = 1.27  $\pm$  0.08  $\mu$ g/mL). Two major constituents, germacrene D (10.31%) and guaiol (13.48%), have been previously reported to possess antioxidant properties. Notably, the essential oil of *Ferula ferulaeoides*, containing guaiol (37%) as the dominant component, also exhibited potent antioxidant activity [21]. These findings suggest that the observed antioxidant potential of *A. monogyna* may be largely attributed to the presence of germacrene D and guaiol, although further studies are required to confirm the contribution of individual compounds.

The essential oil of *A. monogyna* displayed selective antibacterial activity against Gram-positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Lactobacillus fermentum*, with the strongest inhibition observed against *L. fermentum*. In contrast, the oil showed negligible inhibitory effects against Gram-negative strains (*Salmonella enterica*, *Escherichia coli*, and *Pseudomonas aeruginosa*), possibly due to its high content of sesquiterpene hydrocarbons, which are generally less effective against bacteria with complex outer membranes. When compared with other species of the genus *Alphonsea*, a similar trend was observed for Gram-positive bacteria, though differences existed for Gram-negative strains. For instance, *A. madraspatana* exhibited strong inhibitory activity against *E. coli* with MIC and MBC values of 1.56  $\pm$  1  $\mu$ g/mL and 6.25  $\pm$  2  $\mu$ g/mL, respectively [22]. Such variation in antibacterial spectra among *Alphonsea* species may result from differences in their chemical compositions and relative concentrations of bioactive sesquiterpenoids.

Both guaiol and germacrene D, the predominant compounds in *A. monogyna*, have been previously reported for their antibacterial potential. Guaiol shows strong antibacterial effects, particularly against *Mycobacterium* species [23], while germacrene D has demonstrated inhibitory activity against Gram-positive bacteria such as *S. aureus*, *B. subtilis*, and *B. cereus*, as well as moderate activity against Gram-negative bacteria like *E. coli* [24]. However, the moderate concentrations of these bioactive compounds in *A. monogyna* may explain the oil's overall moderate antibacterial potency.

In terms of anti-inflammatory activity, the essential oil significantly inhibited nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages in a concentration-dependent manner. At 100 µg/mL, *A. monogyna* inhibited NO production by 90.74%, which was higher than that of dexamethasone (84.26%). However, at a lower concentration (20 µg/mL), NO inhibition decreased markedly to 16.08%, while dexamethasone retained a relatively high inhibition rate (52.29%). The IC<sub>50</sub> value of *A. monogyna* (56.35 ± 2.57 µg/mL) was higher than that of dexamethasone (14.41 ± 1.55 µg/mL), indicating a moderate level of anti-inflammatory activity. Notably, even at the highest tested concentration (100 µg/mL), the oil maintained high cell viability (92.36%), comparable to that of dexamethasone (99.71%), suggesting minimal cytotoxicity within the tested range. Moreover, previous studies on *Ferula ferulaeoides* revealed its strong anti-inflammatory activity, attributed to guaial (37%), a sesquiterpenoid alcohol identified as the major constituent [21]. Given that guaial is also a dominant component in *A. monogyna*, it may play a similar key role in mediating the oil's anti-inflammatory potential.

Overall, the findings indicate that the leaf essential oil of *A. monogyna* possesses diverse biological activities, including moderate antioxidant, selective antibacterial, and anti-inflammatory effects, with good cellular safety. These results highlight its potential as a promising source of bioactive natural products for pharmaceutical and biological applications.

## 5. CONCLUSION

In conclusion, the leaf essential oil of *A. monogyna* from Hue City contained 47 identified compounds rich in sesquiterpenes, mainly guaial, germacrene D, bulnesol, and bicyclogermacrene. The oil showed moderate antioxidant activity, effective inhibition of nitric oxide production with low cytotoxicity, and selective antibacterial activity against Gram-positive bacteria. These findings provide a comprehensive report on the chemical profile and biological activities of *A. monogyna*, supporting its potential as a promising source of natural agents for pharmaceutical and biological applications.

### Conflict of interest statement

The authors declare no conflict of interest

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